



# Light Scattering Particle Immunoassays in Lab-on-a-Chip for Infectious Agents in Livestock Barns

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## • National Veterinary Science & Quarantine Service, Republic of Korea

### Mission statement

- Quarantine and inspection
- Sanitary control for livestock products
- Animal disease control
- Research and development (in veterinary science)
- Animal medicine
- Animal protection
- Veterinary diagnostics



## • UA-NVRQS International Collaboration

Veterinary diagnostic tool that is:

- Early
- Point-of-care
- Ultra-low detection limit
- Automated

To detect infectious diseases in livestock and poultry:

- Bovine Viral Diarrhea (BVD) - cattle
- Porcine Reproductive and Respiratory Syndrome (PRRS) - swine
- Salmonella - poultry
- Avian influenza (AI) - poultry



## • BVDV & PRRSV

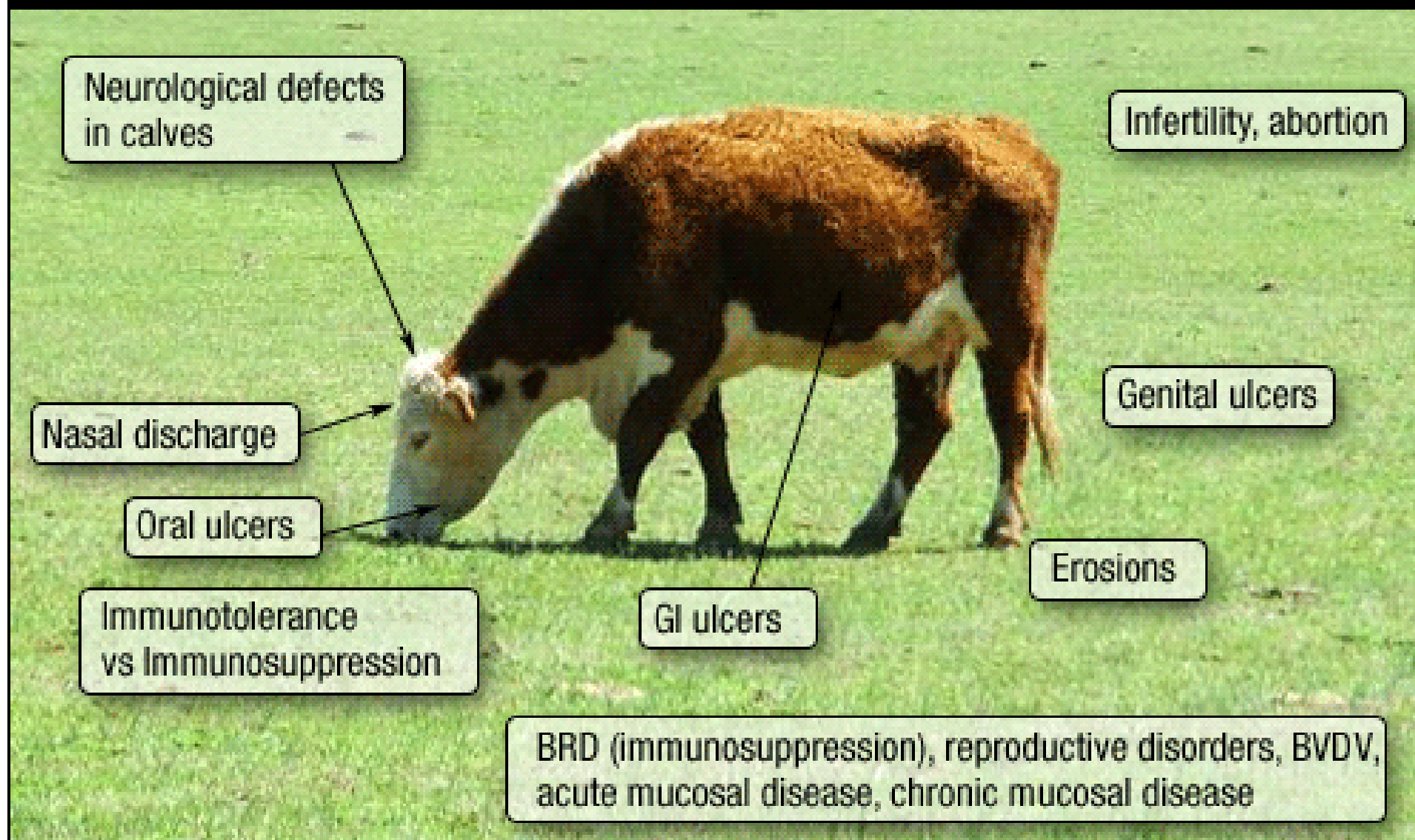
BVDV: one of the most significant viral infections in cattle

- Affects both dairy and beef cattle
- Infected cattle are more susceptible to many respiratory and intestinal pathogens

PRRSV: positive-strand RNA virus

- 50-65 nm (enveloped virus); 25-35 nm (nucleocapsid core)
- Abortions, stillbirths, weak piglets
- Respiratory diseases
- Secondary infections in adult pigs

# BVDV Virus

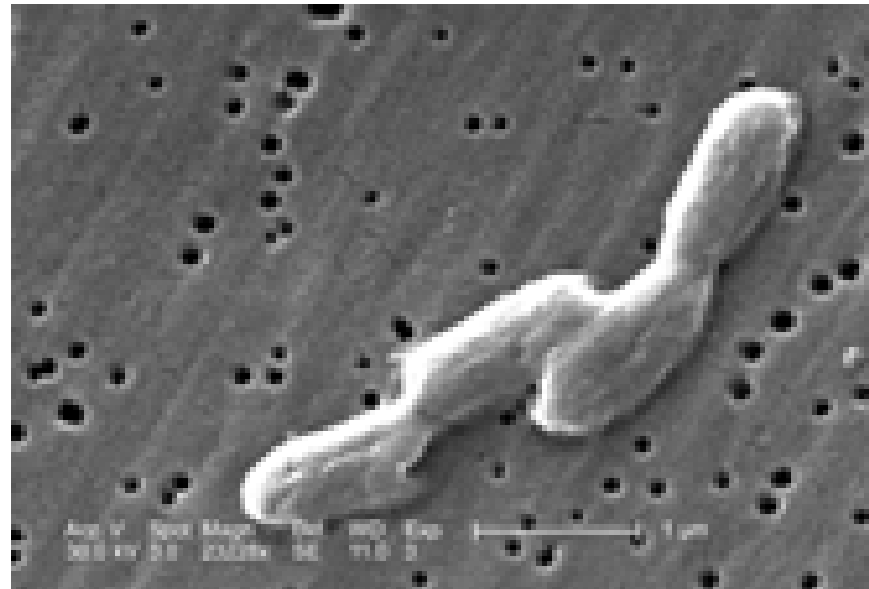




- *Salmonella typhimurium*

*Salmonella typhimurium*: Gram-negative bacteria

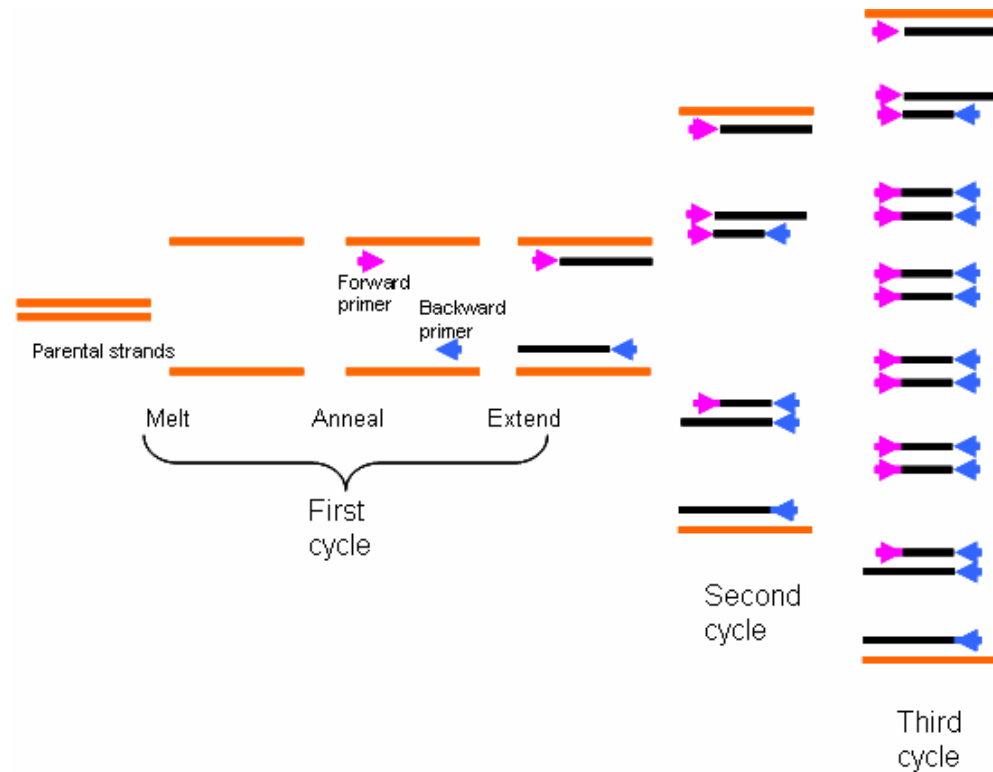
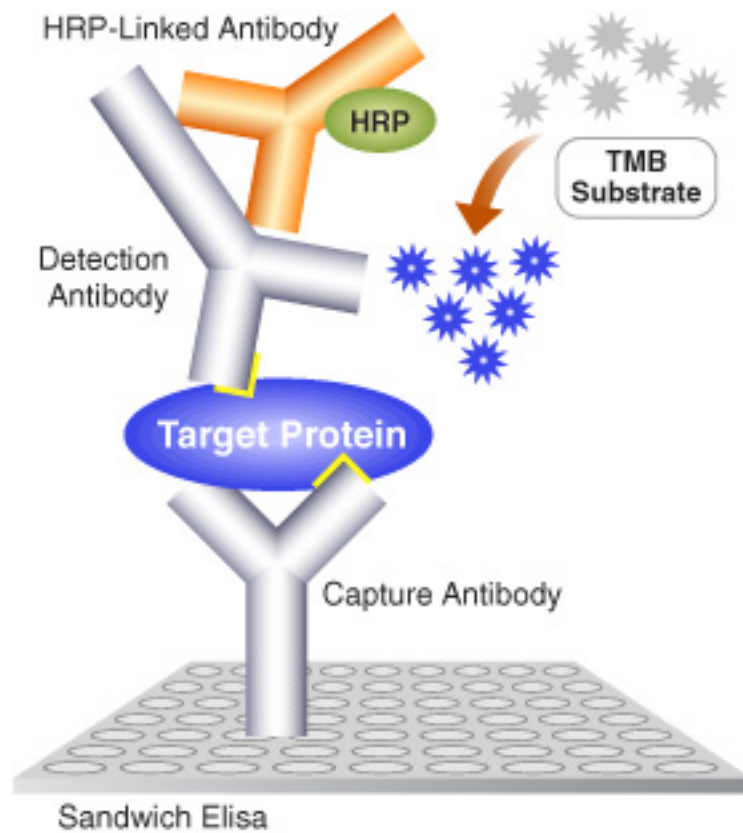
- Cylindrical rod
- 2-3  $\mu\text{m}$  (length); 0.6-0.9  $\mu\text{m}$  (diameter)
- Food poisoning (gastroenteritis)



- Detection limits of current methods

ELISA: 50 pg

PCR: 10-100 TCID<sub>50</sub>/ml



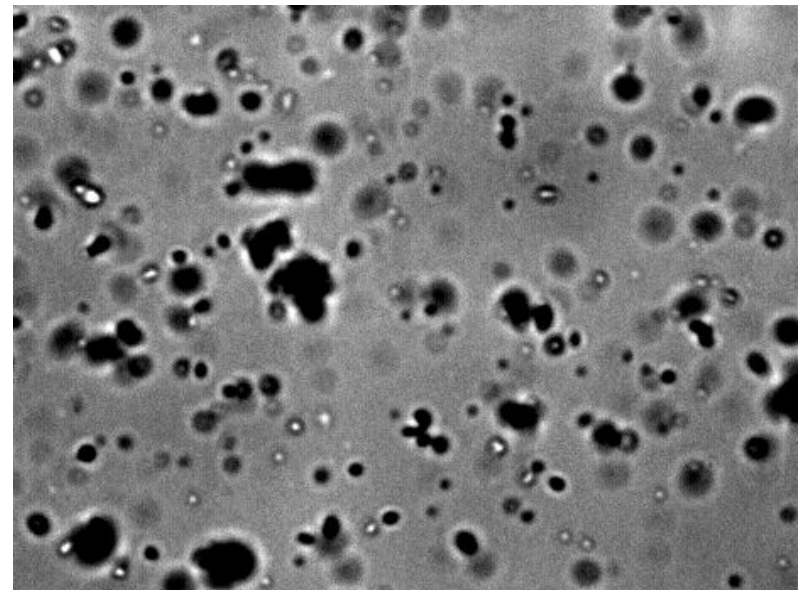
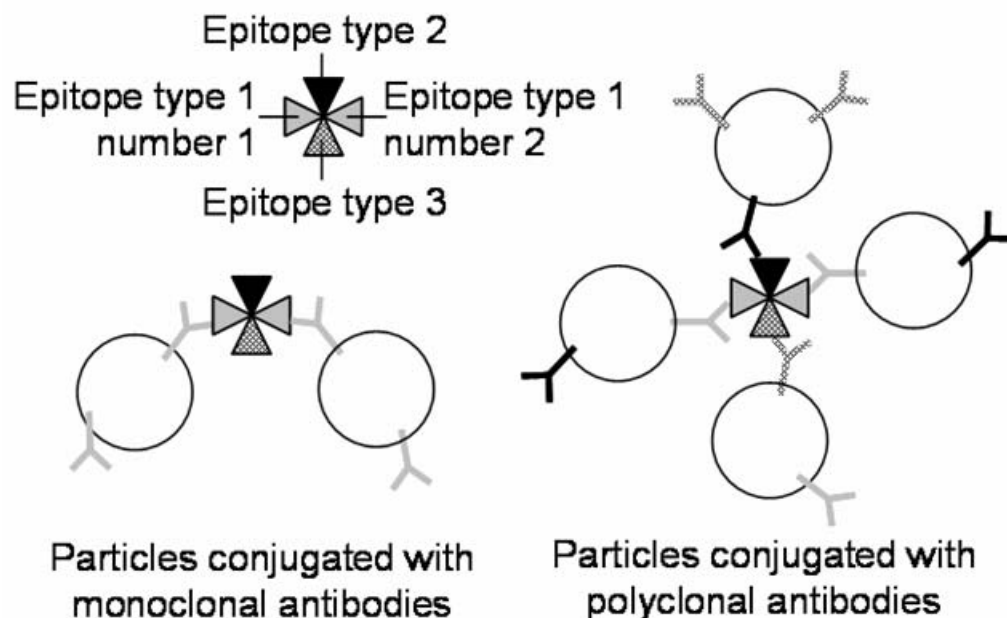


- Lab-on-a-chip (LOC)

- A semiconductor chip used to analyze the composition of a person's body fluids (blood, saliva, urine) for medical purposes. It combines microfluidics and semiconductor processing on the same chip.
- Eliminate human effort such as pipetting.
- Many biological reactions are diffusion-limited; Miniaturization can speed up the reaction.
- Hundreds of assays can be performed simultaneously, saving considerable time and effort.
- We will use LOC for veterinary diagnostics

## • Latex immunoagglutination assay (LIA)

- LIA has NOT been demonstrated in LOC (diffusional mixing issue)
- First demonstrated by UA Biosensors Lab in 2006  
Han, Kim & Yoon, Anal. Chim. Acta 584: 252 (2007)

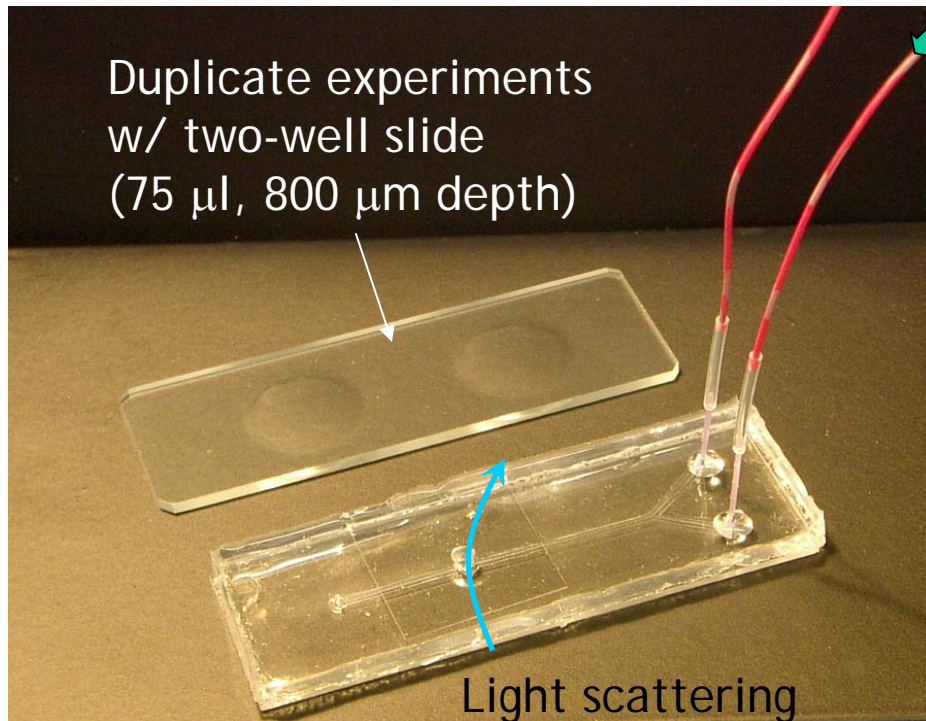


## • Procedure

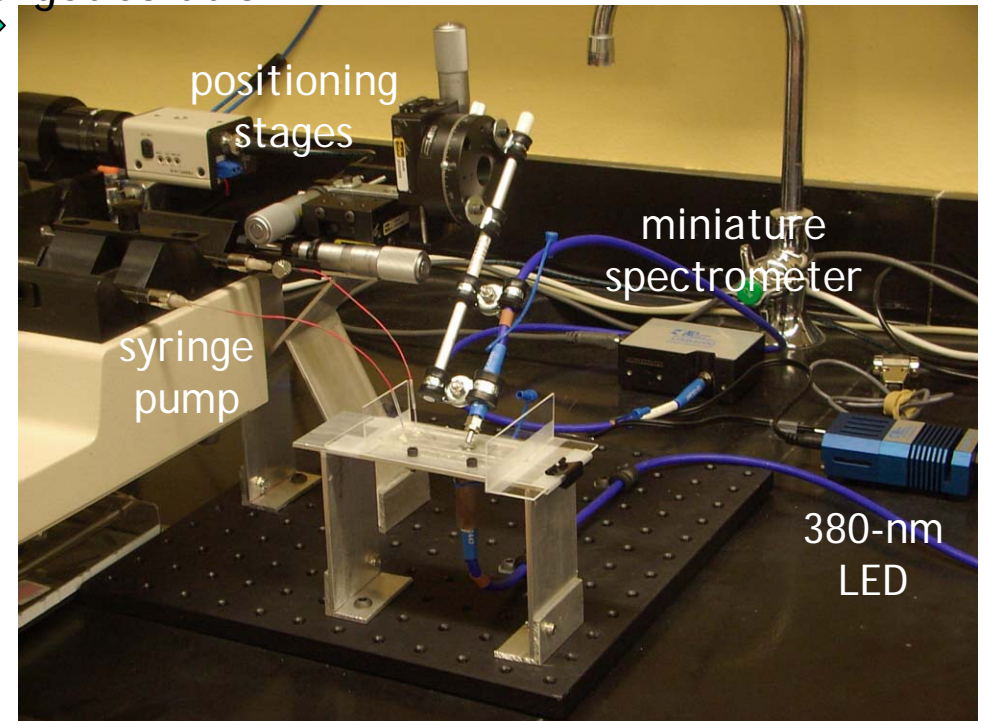
Antibody-conjugated  
structured particles

390-, 510- or 920-nm (mostly 510-nm)  
Highly carboxylated polystyrene particles

Target solution



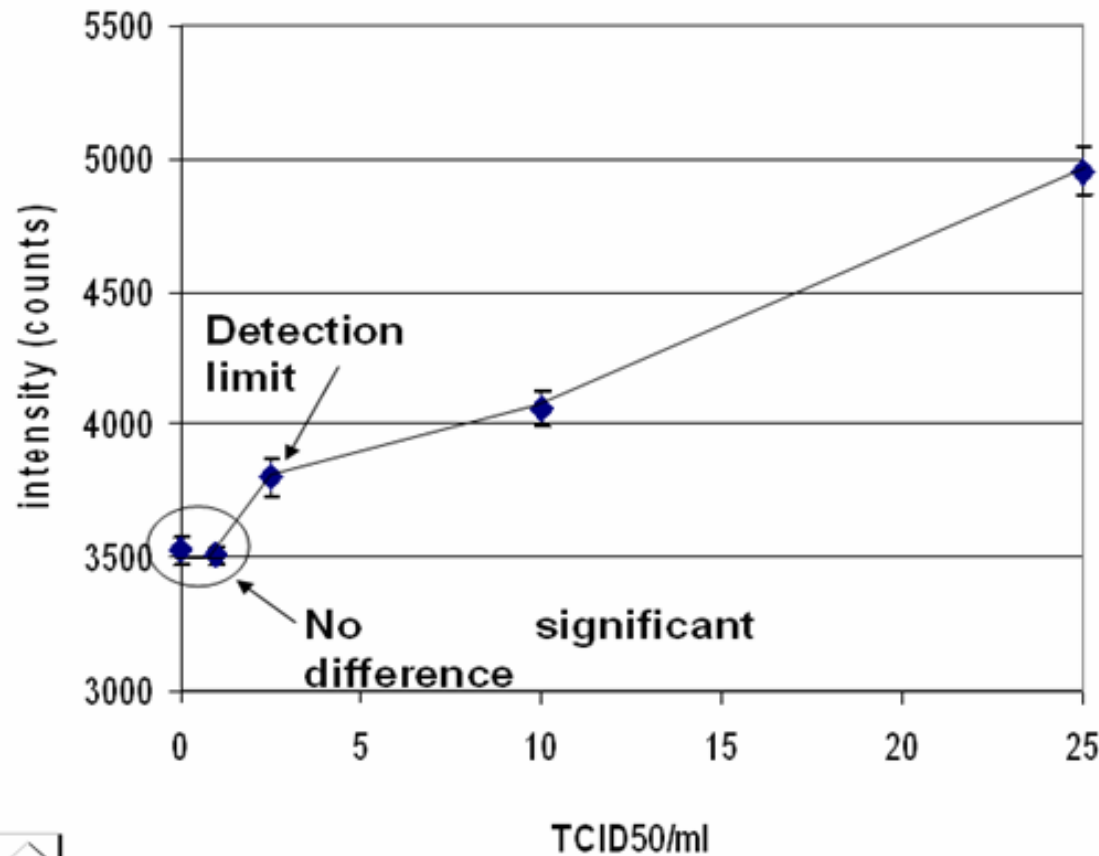
Light scattering  
detection w/ optical fibers



45 degree forward  
light scattering detection

- Bovine Viral Diarrhea Virus (BVDV): 2-well

BVDV in tissue culture media & fetal calf serum

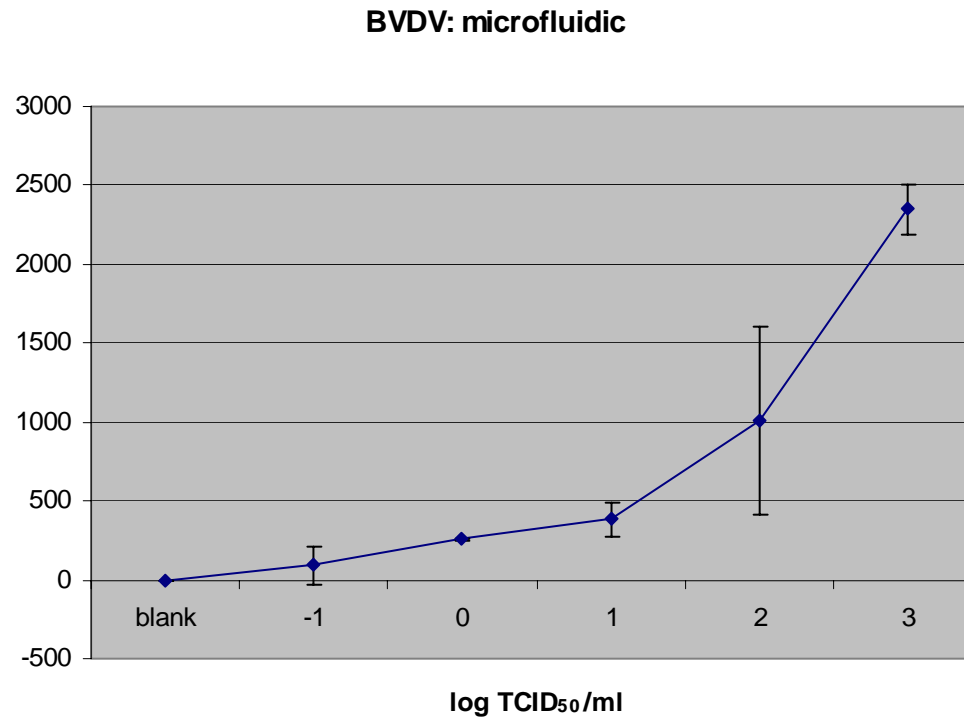


Detection limit  
1~2 TCID<sub>50</sub>/ml

Monoclonal antibody  
Tissue: MDBK  
Washed by centrifuging

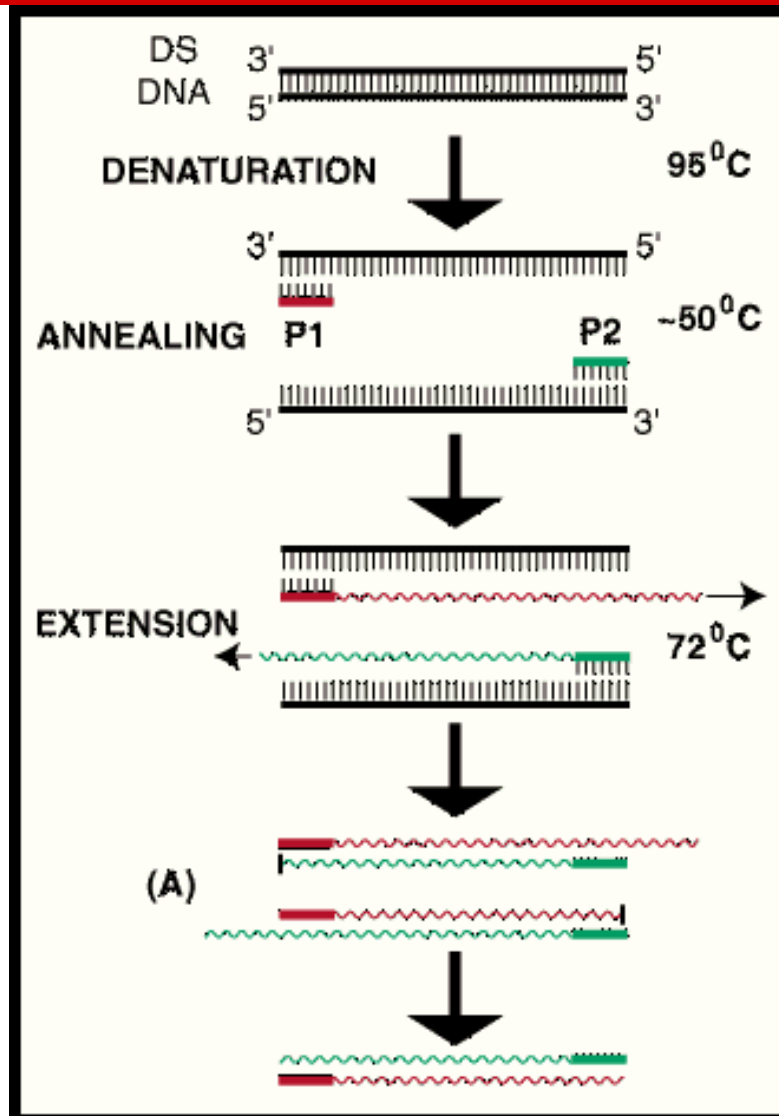
Heinze, Song & Yoon, Proc. SPIE, 6886: 688605 (2008)

- BVDV: microfluidic



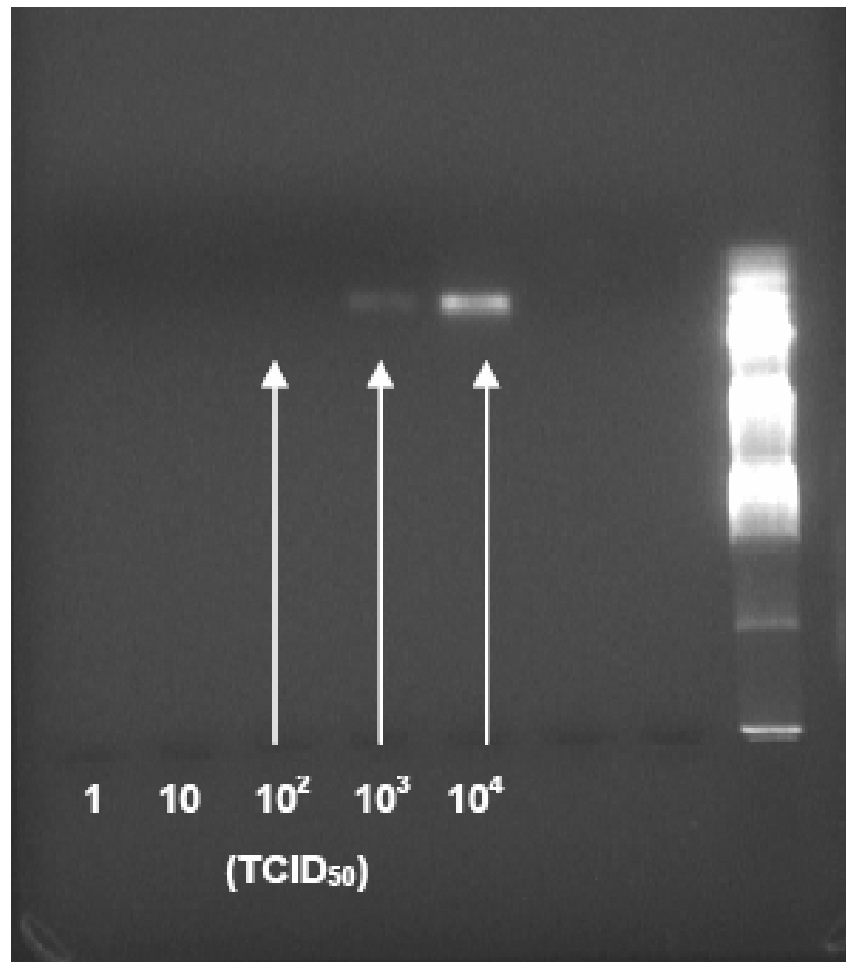
Detection limit: 1 TCID<sub>50</sub>/ml

- BVDV: RT-PCR





- BVDV: RT-PCR



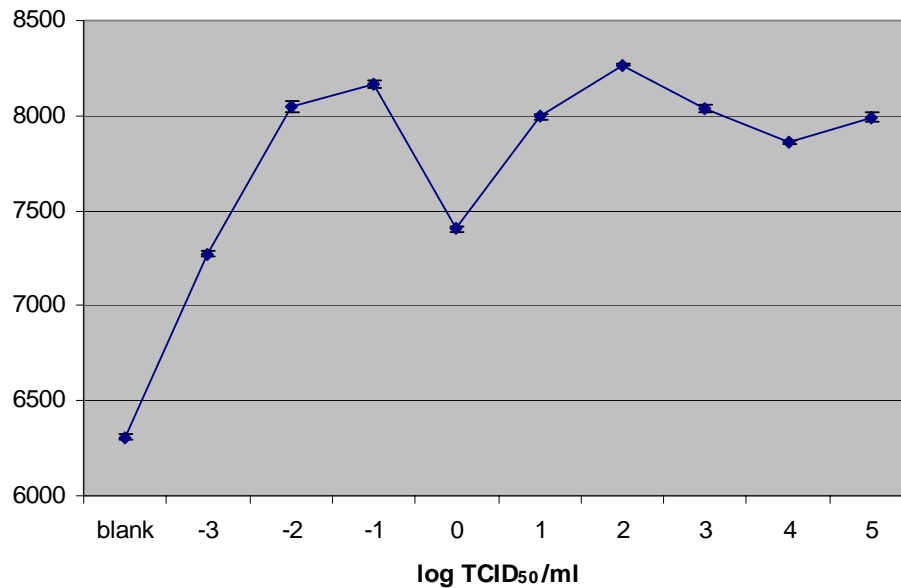
Gel electrophoresis  
of BVDV PCR products  
ranging from 1- $10^4$  TCID<sub>50</sub>/mL

Bands observed  
as low as  $10^3$  TCID<sub>50</sub>/mL

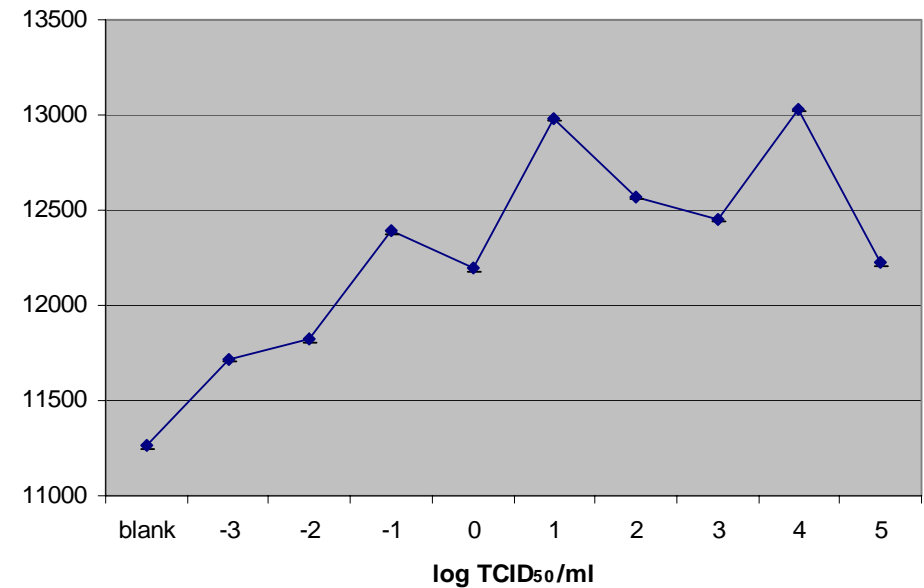
Heinze, Song & Yoon, Proc. SPIE, 6886: 688605 (2008)

- Porcine Reproductive & Respiratory Syndrome Virus (PRRSV): 2-well

PRRSV: 2-well (MAb)



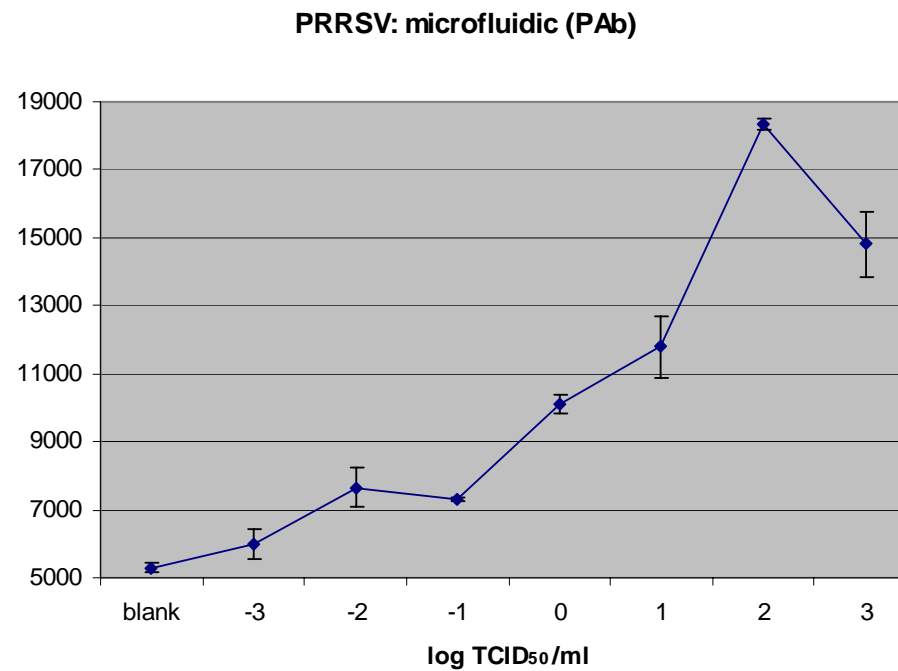
PRRSV: 2-well (PAb)



Live vaccine (attenuated virus)  
Similar to BVDV



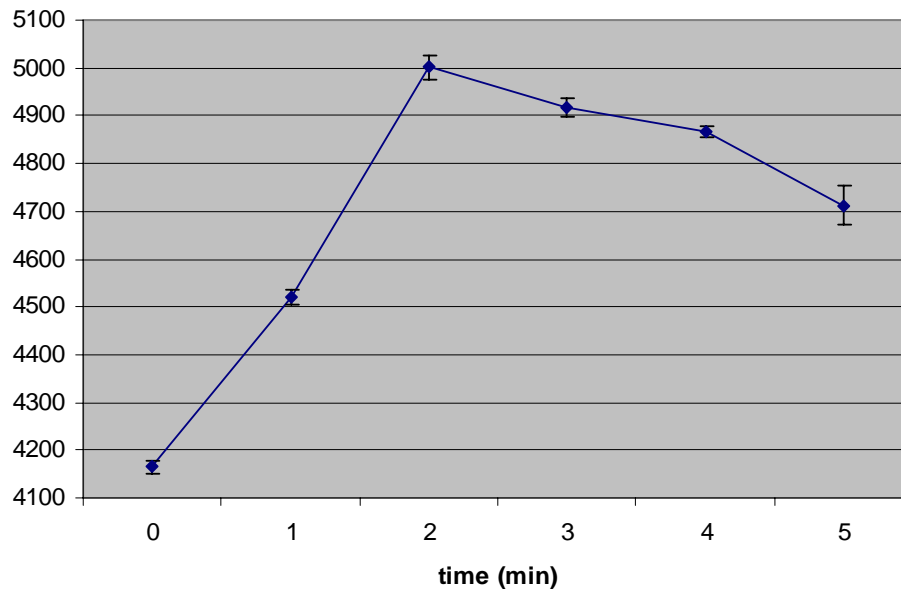
- PRRSV: microfluidic



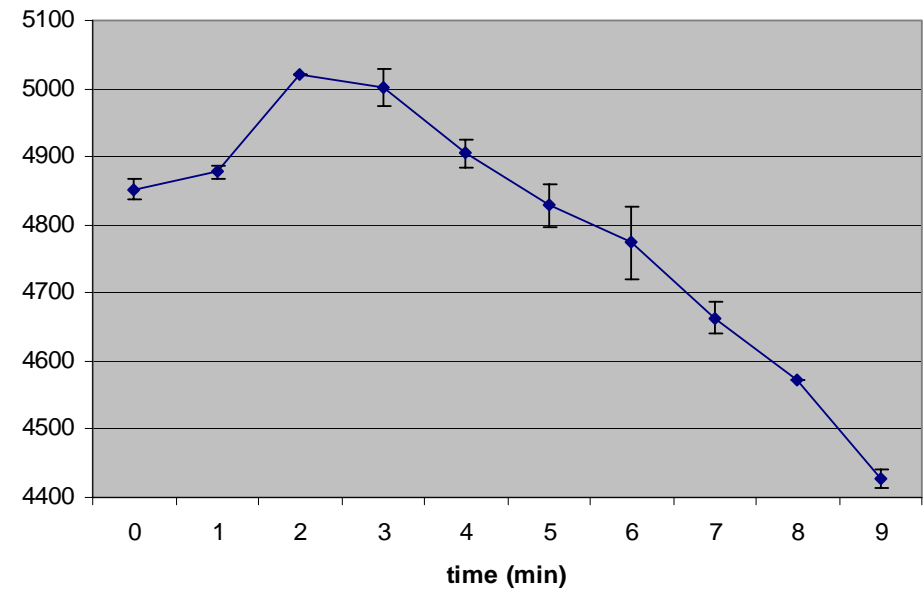
Detection limit:  $10^{-3}$  TCID<sub>50</sub>/ml

- PRRSV: time-course detections

PRRSV: 2-well (PAb)

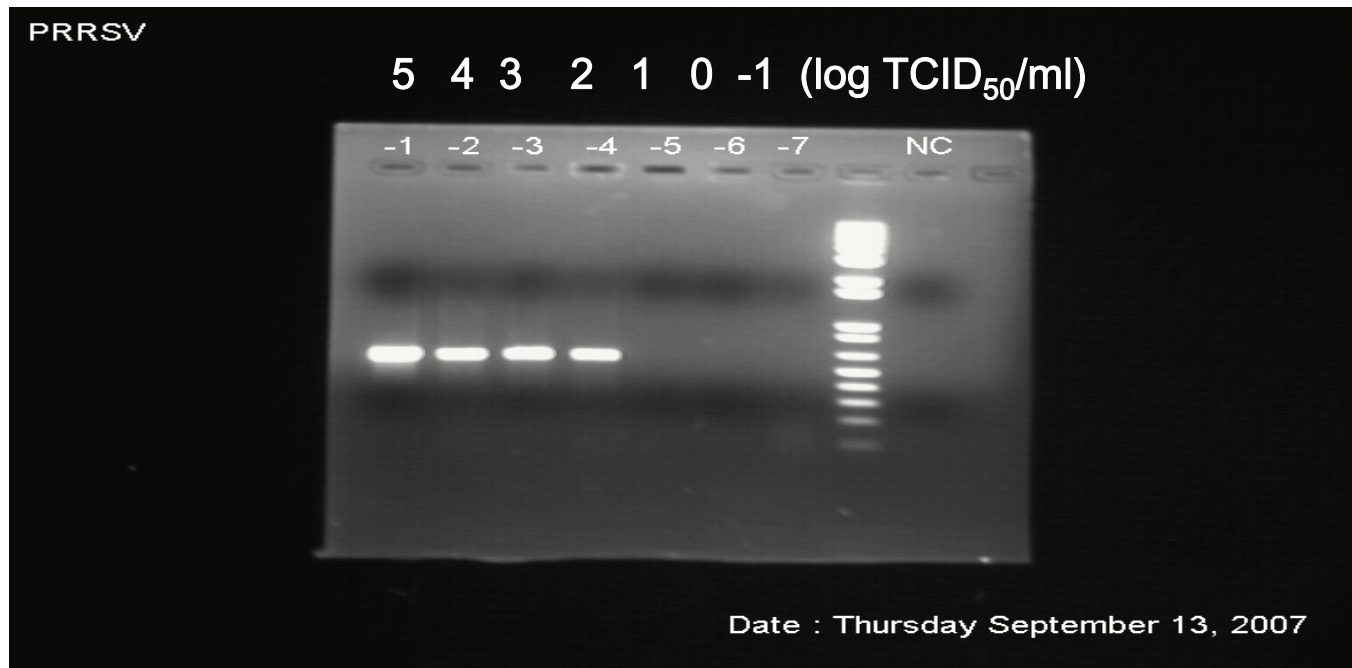


PRRSV: microfluidic (PAb)



Maximum signal at 2 min.

- PRRSV: RT-PCR

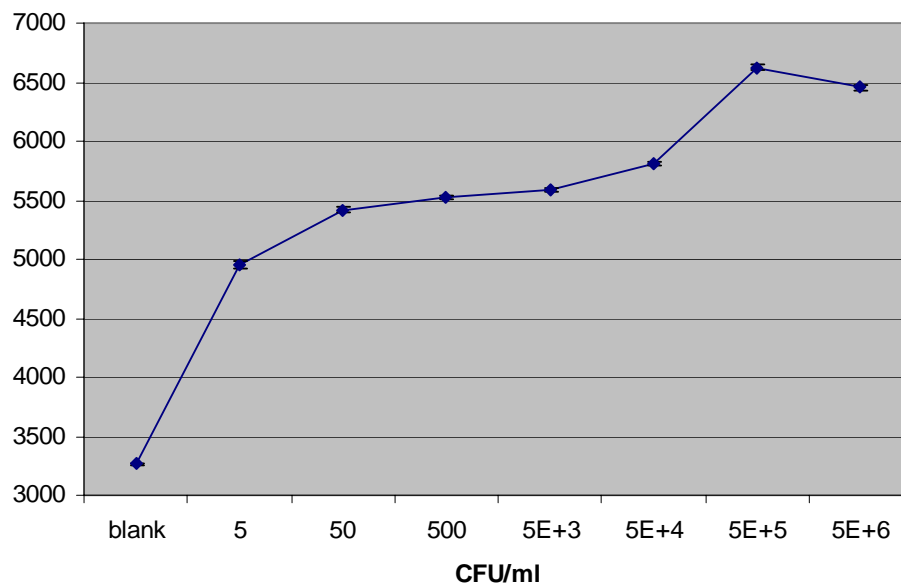


Gel electrophoresis of PRRSV PCR products  
ranging from  $10^{-1}$ - $10^5$  TCID<sub>50</sub>/ml

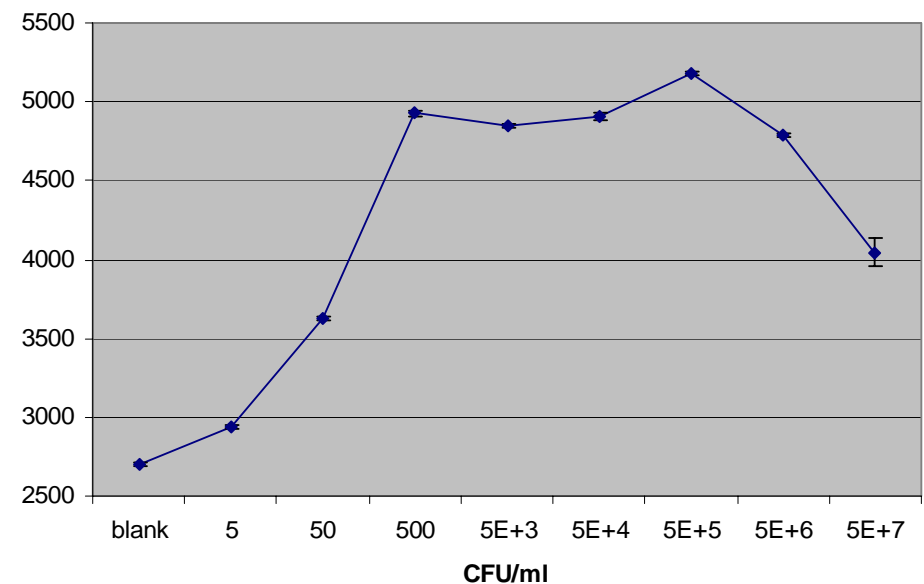
Bands observed as low as  $10^2$  TCID<sub>50</sub>/ml

- Salmonella typhimurium: 2-well

Salmonella: 2-well (MES, KPL antibody)



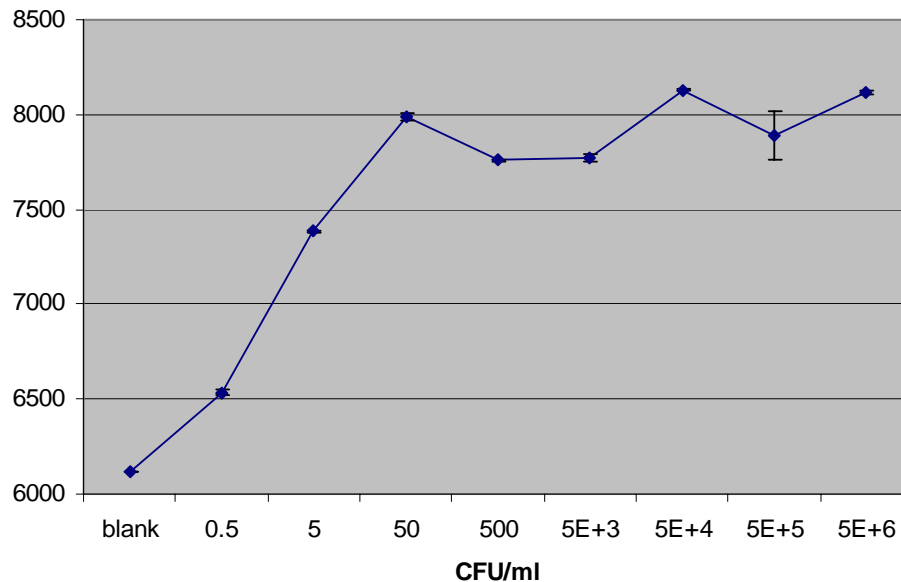
Salmonella: 2-well (PBS, KPL antibody)



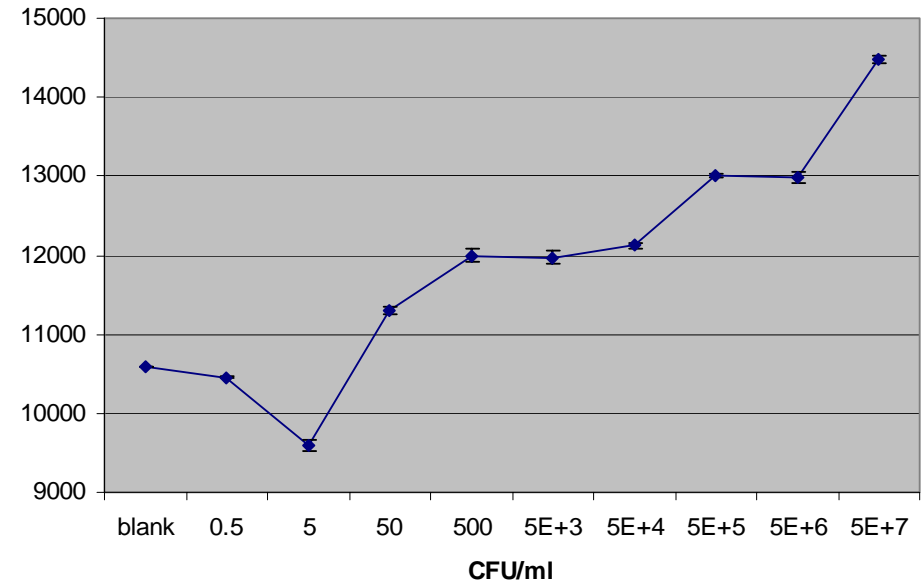
Cultured in media, washed by centrifuge  
Polyclonal antibodies

- Salmonella: 2-well & microfluidic

Salmonella: 2-well (PBS, Biodesign antibody)

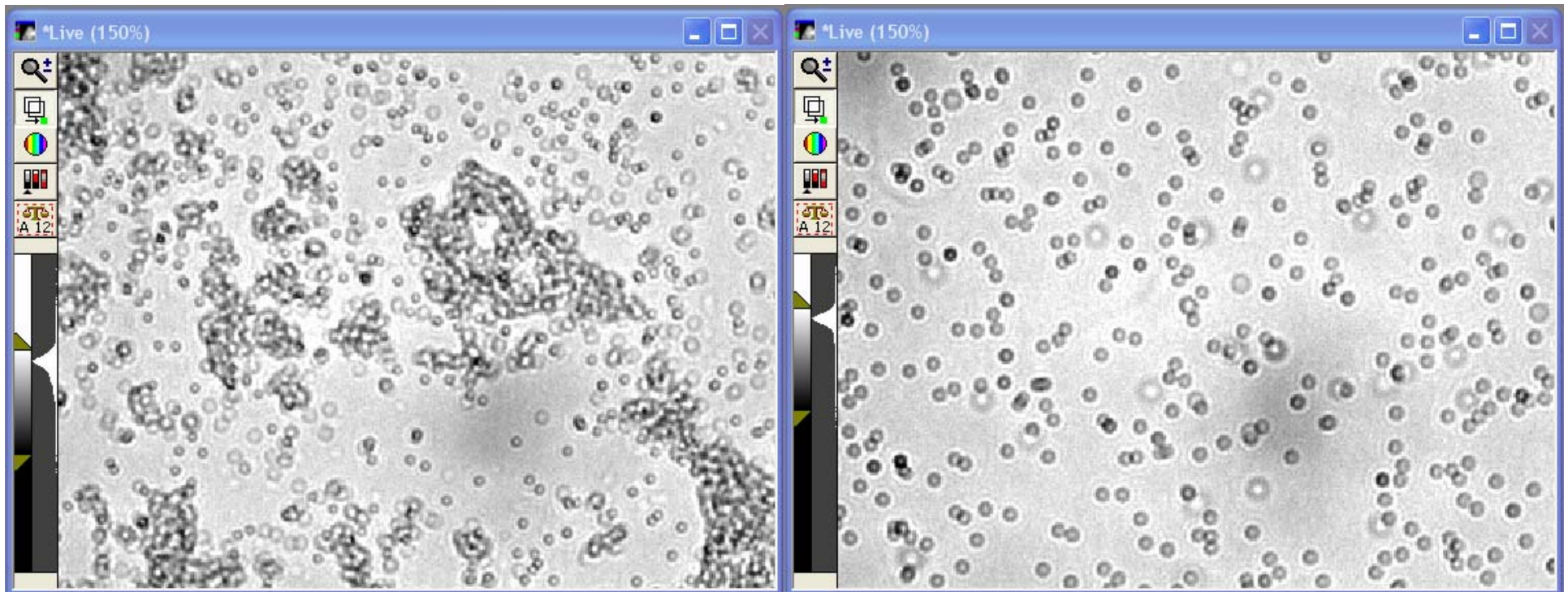


Salmonella: microfluidic



Detection limit: 0.5-50 CFU/ml

- Salmonella: immunoagglutination images with 910-nm beads





- Other targets under testing

- *Brucella abortus*
- Influenza A (H3N2)



## • Conclusion

- Detection limits: BVDV  $<1$  TCID<sub>50</sub>/ml (MAb)  
PRRSV  $10^{-3}$  TCID<sub>50</sub>/ml (PAb)  
Salmonella  $<5$  CFU/ml (PAb)
- Maximum signal achieved at 2 min
- Total assay time  $<5$  min

## • Future work

- Integrate optical fibers (optical waveguide)
- Replace miniature spectrometer with Avalanche photodiode (APD) and computer/software with op-amp circuit/voltmeter
- Test for field sample





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